Research Article

DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE ESTIMATION OF DISOPYRAMIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTARCT
A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Disopyramide in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Methanol: Acetonitrile: THF in the ratio of 55 : 45 : 5 ,(v/v/v) was used. The flow rate was 1.0ml/min and effluents were monitored at 265nm. The retention time for Disopyramide was 2.967 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.03ppm and 0.099ppm respectively and recovery of Disopyramide from tablet formulation was found to be 98.87%. The proposed method was successfully applied for the quantitative determination of Disopyramide in tablet formulation.

Keywords: Disopyramide, HPLC, Linearity, Validation.

INTRODUCTION
Disopyramide (INN, trade names **Norpace** and **Rythmodan**) is an antiarrhythmic medication. It is a Class Ia antiarrhythmic (sodium channel blocker) used in the treatment of ventricular tachycardias.
It has no effect on alpha or beta adrenergic receptors. It resembles Quinidine but it has a marked anti-muscarinic effect on the heart, for this reason, it is not considered as a drug of 1st choice. It is also used in ventricular arrhythmia and supraventricular arrhythmia that might follow myocardial infarctions.

Molecular formula $C_{21}H_{29}N_3O$
   Molecular weight   339.475 g/mol
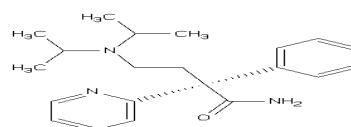
Fig. 1: Molecular Structure of Disopyramide

Literature survey revealed that numerous methods have been reported for estimation of Disopyramide in pharmaceutical formulations has been reported[1-13].
Present study involves development of LC method using simple mobile phase which is sensitive and rapid for quantification of Disopyramide in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines.

EXPERIMENTAL
Instrument
The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and materials
Methanol of HPLC grade was purchased from E.Merck, Mumbai, India. LC grader water was obtained by double distillation and purification through milli – Q water purification system. Ortho phosphoric acid of analytical grade was procured from qualigens, Mumbai,India.

Preparation of Standard Stock Solution
A stock solution of Disopyramide was prepared by accurately weighing 10mg of drug, transferring to 100ml of volumetric flask, dissolving in 25ml of solvent and diluting up to mark with solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 2.5ppm of Disopyramide. Resultant solution was filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper.

Preparation of sample Solution
The formulation tablets of Disopyramide were crushed to give finely powdered material. Powder equivalent to 10mg of Disopyramide was taken in 10 ml of volumetric flask containing 5ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 2ppm.

Chromatographic conditions
The mobile phase consisting of Methanol: Acetonitrile: THF were filtered through 0.45µ Ultipor N_{66} Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 55:45:5 ,v/v/v

and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 265nm with a run time of 6min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.
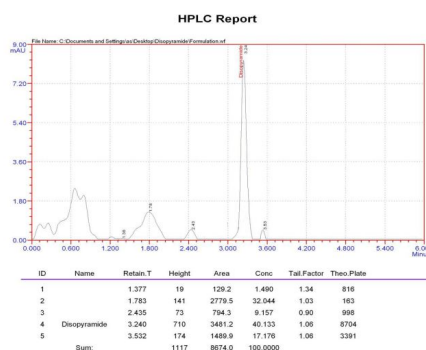

ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		1.377	19	129.2	1.490	1.34	816
2		1.783	141	2779.5	32.044	1.03	163
3		2.435	73	794.3	9.157	0.90	998
4	Disopyramide	3.240	710	3481.2	40.133	1.06	8704
5		3.532	174	1489.9	17.176	1.06	3391
	Sum:		1117	8674.0	100.0000		

Fig. 2: HPLC chromatogram of Disopyramide formulation

Calibration curve
Appropriate aliquots of standard Disopyramide stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 0.5, 1, 1.5, 2 and 2.5ppm of Disopyramide. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Disopyramide was constructed by plotting peak area ratio versus applied concentration of Disopyramide and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Disopyramide in tablet sample was found out using regression equation.

Method validation
The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

Accuracy
The accuracy of the method was determined by calculating recovery of Disopyramide by

the method of standard addition. Known amount of Disopyramide (1ppm, 0.5ppm and 1.5ppm) was added to a pre quantified sample solution and the amount of Disopyramide was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Disopyramide was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision
The intra-day precision study of Disopyramide was carried out by estimating the correspondence responses six times on the same day with 2ppm concentration and inter-day precision study of Disopyramide was carried out by estimating the correspondence responses six times next day with 2ppm concentration.

Linearity and range
The linearity of the method was determined at six concentration levels ranging from 0.5-2.5ppm for Disopyramide.

Specificity
Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification
Limit of detection = 0.03ppm
Limit of quantification = 0.099ppm

Stability
In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness
Robustness of the method was studied by changing the composition of organic phase by ±5% and the P^H by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION
The UV spectra of Disopyramide showed that the drug absorbs appreciably at 265nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol: Acetonitrile: THF in the ratio of 55:45:5, (v/v/v) . The retention time of Disopyramide was found to be 2.967 min, which indicates a good base line.
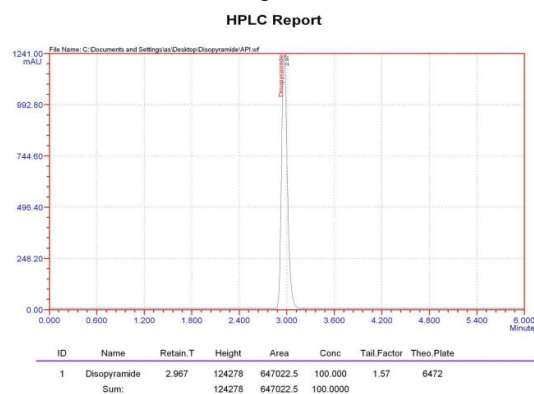

Fig. 3: HPLC chromatogram of Disopyramide

The number of theoretical plates was found to be 6471.60, which indicates efficient performance of the column. The asymmetric factor was found to be 1.57, which indicates asymmetric nature of the peak. The calibration curve for Disopyramide was obtained by plotting the peak area ratio versus the concentration of Disopyramide over the range of 0.5-2.5ppm, and it was found to be linear with r^2=0.999. The regression equation of Disopyramide concentration over its peak area ratio was found to be y = 1597.01+ 80675.14x, where x is the concentration of Disopyramide (ppm) and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantitation for Disopyramide was found to be 0.03ppm

and 0.099ppm, indicates the sensitivity of the method. The system suitability and validation parameters were given in table 2. The high percentage of recovery of Disopyramide was found to be 98.87% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Disopyramide in tablet formulation. The result for Disopyramide was comparable with a corresponding labeled amount (Table 3). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Table 1: Regression analysis of the calibration curve

Parameters	Values
Calibration range (ppm)	0.5-2.5
Slope	80675.14
Intercept	1597.01
Correlation coefficient (r^2)	0.999

Table 2: System suitability and validation parameters

Parameters	Results
Theoretical plates (N)	6471.60
Retention time (min)	2.967
Asymmetric factor	1.57
LOD (ppm)	0.03
LOQ (ppm)	0.099
Accuracy (%)	99.35
R.S.D. (%)	0.900

Table 3: Assay results of tablet formulation

Formulation	Labelled claim (mg)	% of Disopyramide in Tablet
Regubeat	100	14.28

CONCLUSION
Proposed study describes new LC method for the estimation of Disopyramide in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Disopyramide in its tablet formulation and serum.

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